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Short sequence-paper

Cloning and characterization of a cotton lipid transfer protein gene specifically expressed in fiber cells [1]

Din-Pow Ma [a,*], Hsi-Chou Liu [a], Hingpoh Tan [a], Roy G. Creech [b], Johnie N. Jenkins [b,c], Yung-Fu Chang [d]

[a] *Department of Biochemistry and Molecular Biology, Box 9650, Mississippi State University, Mississippi, MS 39762 USA*
[b] *Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS 39762, USA*
[c] *Crop Science Research Laboratory, USDA / ARS, Mississippi State, MS 39762, USA*
[d] *Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*

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Abstract

A cotton genomic library was screened using a fiber-specific cDNA (GH3) encoding a lipid transfer protein (LTP). One genomic clone (1.7 kb DNA insert) containing the *Ltp* gene (*Ltp*6) was sequenced and characterized. The *Ltp*6 contains an open reading frame of 360 bp, which is interrupted by a single intron (136 bp) located in the region corresponding to the C-terminal of the protein. The derived amino-acid sequence of LTP6 is 64% homologous to that of GH3. Like the GH3 gene, the *Ltp*6 is specifically expressed in fiber cells in a temporal manner. However, its expression level is lower than that of GH3.

Keywords: cDNA; Genomic library; Intron; Northern analysis; Nucleotide sequence

Plant lipid transfer proteins (LTPs) are a group of small, basic proteins containing seven to eight conserved cysteine residues. Previously, they were thought to be of cytoplasmic origin and participate in intracellular transport of phospholipids for in vivo membrane synthesis [1]. However, the presence of a signal peptide in all sequenced plant *Ltp* cDNAs and genes and the localization of LTPs in the cell wall [2] and outer cellular layers [3–6] suggest that they are extracellular proteins. By using a differential screening method, we had previously isolated a cotton fiber-specific cDNA (GH3) encoding LTP [7]. The GH3 gene is expressed at high levels in the primary cell wall synthesis stage during fiber development, and the fiber LTP may be involved in cutin synthesis. To further study the fiber LTP and its expression, the GH3 was used as a hybridization probe to retrieve homologous genomic clones.

Cotton nuclear DNA was isolated from *Gossypium hirsutum* L. cultivar DES119 according to Paterson et al. [8]. A genomic library, constructed by cloning *Eco*RI-digested genomic DNA fragments into lamba gt10, was screened by hybridization using GH3 as a probe. One positive clone containing a 1.7 kb insert was isolated and sequenced by the dideoxy chain

* Corresponding author. Fax: +1 (601) 3258664; E-mail: dm1@ra.msstate.edu

[1] The nucleotide sequence data reported will appear in the GenBank Nucleotide Sequence Databases under the accession number U64874.

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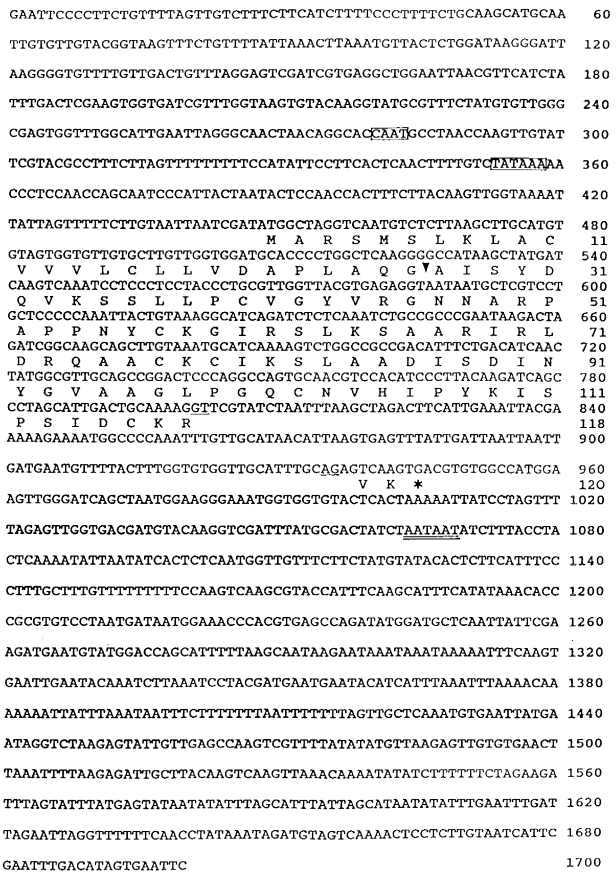

Fig. 1. Nucleotide sequence of the cotton *Ltp*6 gene. The deduced amino-acid sequence is shown below its nucleotide sequence. Promoter elements are boxed. The conserved intron splicing sequences GT and AG are underlined. The double underline represents the polyadenylation signal. The signal peptide cleavage site is marked with an arrowhead.

termination method [9]. The 1.7 kb *Eco*RI fragment contains a *Ltp* gene (*Ltp*6) encoding a LTP of 120 amino acids (Fig. 1). The derived LTP protein (LTP6) has the general characteristics of plant LTP. It has a low molecular weight (12 802) and a high (basic) isoelectric point (p*I* 9.8), and contains a low content of aromatic amino acids and high percentage of proline, leucine, valine, alanine, and serine. The protein also contains a secretion signal peptide (26 amino acids) at the N-terminal. However, only six conserved cysteine residues are found in the mature LTP6. The Cys at amino-acid positions 30 and 56 found in all known plant LTP is absent in LTP6, which instead has a Tyr at both positions. Similar to other plant *Ltp* genes, the open reading frame of

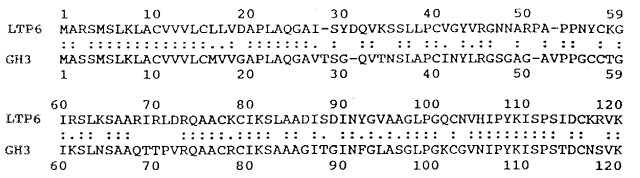

Fig. 2. Amino-acid sequence comparison of GH3 and LTP6 proteins. Identical and chemically similar amino acids are denoted by double and single dots, respectively.

*Ltp*6 is interrupted by a single intron of 136 bp located in the region corresponding to the C-terminal of the protein. The intron has typical GT and AG sequences at the 5′ and 3′ splicing sites, respectively. The 5′ flanking region of the *Ltp*6 gene contains TATA and CAAT boxes which would act as promoter elements for its transcription. A putative polyadenylation signal AATAAT can also be located at the 3′ flanking sequence. The LTP6 has a 64% identity in amino-acid sequence when compared with that of GH3 (Fig. 2). Homologous sequences between the two LTPs are mostly found at N- and C-terminal regions.

Northern analysis was used to determine *Ltp*6 expression in various cotton tissues including leaves, roots, flowers, and fibers (Fig. 3). Using the 1.7 kb *Ltp*6 genomic DNA as a probe, a 0.75 kb transcript was detected in fibers only and its level reached the highest at 15 DPA (days postanthesis). These results indicate that the *Ltp*6 gene has an expression pattern similar to that of the GH3 gene; however, its expression level is much lower than that of GH3 (Fig. 3).

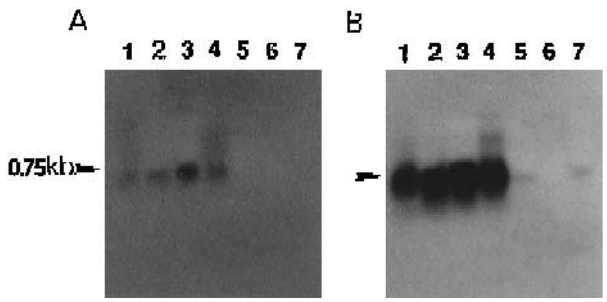

Fig. 3. Northern analysis of the *Ltp*6 gene and GH3 cDNA. Total RNAs (10 μg) from developing fiber (lanes 1, 5 DPA; lane 2, 10 DPA; lane 3, 15 DPA; lane 4, 20 DPA), leaf (lane 5), root (lane 6), and flower (lane 7) were hybridized with *Ltp*6 (A). The *Ltp*6 probe was removed and the blot was rehybridized with the GH3 probe (B).

Since the cultivated cotton is an allotetraploid species, it is not surprising to detect the expression of two related *Ltp* genes.

Plant *Ltp* genes are generally expressed in a spatial and temporal pattern. Tissue-specific expression of LTP has been found in tobacco [10,11], *Arabidopsis* [2], castor beans [12], barley [3,4,13–15], maize [16], and *Brassica* [17]. In general, high levels of *Ltp* mRNA were detected in epidermal cells. LTP expression was also induced by salt, heat, and drought stress [18,19]. Recently, it has been shown that plant LTPs are growth inhibitors of bacteria and fungal pathogens [20,21]. We have previously reported that a cDNA clone (GH3) encoding a LTP is developmentally regulated and differentially expressed in fiber cells, and have proposed the possible function of GH3 LTP protein being involved in cutin synthesis during fiber development [7]. The participation of LTP in the synthesis of cutin and cuticular wax has also been proposed by Thoma et al. [22], Hendriks et al. [23], and Pyee and Kolattukudy [24]. It is interesting that a GH3 LTP homolog, LTP6, is also expressed in fibers in a similar pattern as the GH3, although its expression level is lower. This observation suggests that different forms of LTPs (GH3 and LTP6) might be needed for fiber cell elongation during the primary cell wall synthesis stage. Structural differences between GH3 and LTP6 are obvious in hydrophobicity plots (data not shown) and numbers of disulfide bond formation between Cys residues. Using X-ray crystallography, Shin et al. [25] have shown that the eight conserved cysteine residues of a mature maize LTP form four disulfide bridges: Cys^{4}-Cys^{52}, Cys^{14}-Cys^{29}, Cys^{30}-Cys^{75}, and Cys^{50}-Cys^{89}. The three-dimensional structure of the maize LTP has a tunnel-like hydrophobic cavity which would allow accommodation of a long fatty acyl chain. Since the mature LTP6 lacks Cys at amino-acid positions 4 and 29, it will be interesting to determine how LTP6 would fold into a tertiary structure with the presence of only two possible disulfide bonds. It is proposed that GH3 has a stronger fiber promoter than that of *Ltp*6 based on Northern analyses. Attempts to clone the GH3 corresponding *Ltp* gene are currently under way.

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